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**Identification of diverse archaeal proteins with class III signal peptides cleaved by distinct archaeal prepilin peptidases**

Szabó, Zalán; Oliveira Stahl, Adriana; Albers, Sonja-V.; Kissinger, Jessica C.; Driessen, Arnold J.M.; Pohlschröder, Mechthild; Pohlschroder, M.

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Table S2. Composition of operons containing FlaFind positive genes

Extent of gene locus	Number of genes	Number of substrates	<i>fla*</i>	<i>tadA</i>	<i>tadC</i>	<i>flaK</i>	<i>eppA</i>	Other proteins encoded by operon/additional comments
<b>Crenarchaeota</b>								
<b><i>Aeropyrum pernix</i>K1</b>								
APE0697-APE0695	3	1		✓				
APE1907-APE1895	8	2	✓	✓	✓			
<b><i>Pyrobaculum aerophilum</i> str. IM2</b>								
PAE2394-PAE2414	16	2		✓				
<b><i>Sulfolobus acidocaldarius</i> DSM 639</b>								
Saci_1178-Saci_1172	7	1	✓	✓	✓			
Saci_1493-Saci_1500	9	2 <sup>†</sup>		✓	✓			Predicted EndoIII-related nuclease (COG0177), Lhr-like helicase (COG1201), Glycosyltransferase probably involved in cell wall biogenesis (COG1215), Predicted transcriptional regulators (COG1475)
<b><i>Sulfolobus solfataricus</i> P2</b>								
SSO0122-SSO0117	6	2		✓	✓			tRNA and rRNA cytosine-C5-methylases (COG0144)
SSO2323-SSO3215	8	1	✓	✓	✓			operon disrupted by IS element integration
SSO2686-SSO2678	7	2		✓	✓			
SSO3142-SSO3138	5	3						
<b><i>Sulfolobus tokodaii</i> str. 7</b>								
ST1074-ST1070	5	3						
ST1396-ST1400	5	2		✓	✓			
ST2518-ST2524	7	1	✓	✓	✓			
<b>Euryarchaeota</b>								
<b><i>Archaeoglobus fulgidus</i> DSM 4304</b>								
AF0763-AF0758	6	4						
AF1055-AF1037	19	2	✓	✓	✓			Chemotaxis signal transduction system (AF1046-AF1037)
<b><i>Haloarcula marismortui</i> ATCC 43049</b>								
rrnAC1252-rrnAC1244	9	5		✓	✓			Aspartate/tyrosine/aromatic aminotransferase (COG0436)
rrnAC1951-rrnAC1945	6	3		✓	✓			
rrnAC2203-2183	16	1	✓	✓	✓			Chemotaxis signal transduction system (rrnAC2194-

								rrnAC2192)
rrnAC1420-rrnAC1423	5	2						One gene located between rrnAC1422 and rrnAC1423 (similar to rrnAC1245) not annotated in published sequence
rrnAC1425-rrnAC1424	2	2						
<b><i>Halobacterium sp.</i> NRC-1</b>								
VNG0200C-VNG0198H	7	3						one gene located between VNG0204H and VNG0200C (similar to rrnAC1948) not annotated in published sequence
VNG0962G -VNG0953C	8	3 <sup>†</sup>	✓					
VNG1008G-VNG1009G	2	2	✓					
<b><i>Methanocaldococcus jannaschii</i> DSM 2661</b>								
MJ0836-MJ0832.1	7	2					✓	Part of split operon, see Figure 3
MJ0891-MJ0906	16	3	✓	✓	✓	✓		
MJ1291 -MJ1282.1	12	1		✓	✓	✓		
MJ1467-MJ1472	7	2 <sup>†</sup>						Part of split operon, see Figure 3
<b><i>Methanococcus maripaludis</i> S2</b>								
MMP0231-MMP0241	11	3					✓	See Figure 3
MMP0602-MMP0599	4	2						Orotidine-5'-phosphate decarboxylase (COG0284), Precorrin-6x reductase (COG2099)
MMP1666-MMP1676	11	3	✓	✓	✓			
<b><i>Methanopyrus kandleri</i> AV19</b>								
MK0704-MK0698	7	2					✓	upstream genes not directly linked to this locus encode chaperone-like ATPase (COG0606), Flp pilus assembly ATPase (COG4962) and two TadC homologs (COG2064)
MK0696-MK0694	3	1						gene cluster possibly co-regulated with above listed operon
<b><i>Methanosarcina acetivorans</i> C2A</b>								
MA1234-MA1230	5	3						ABC transporter components
MA3062-MA3054	9	2	✓	✓	✓			
MA3077-MA3084	8	1	✓	✓	✓			second <i>fla</i> operon similar to MM0418-MM0411
MA3093-MA3098	6	1		✓	✓			ABC transporter components
<b><i>Methanosarcina mazei</i> Go1</b>								

MM0323-MM0315	9	2	✓	✓	✓			upstream gene locus codes for chemotaxis system
MM0418-MM0411	8	1	✓	✓	✓			second <i>fla</i> operon similar to MA3077-MA3084
<b><i>Methanothermobacter thermautotrophicus str. Delta H</i></b>								
MTH421-MTH432	12	4 <sup>†</sup>					✓	conserved membrane protein (COG4025), predicted PP loop family ATPase (COG2102)
<b><i>Pyrococcus abyssi</i> GE5</b>								
PAB1376-PAB1388	13	3	✓	✓	✓			SAM dependent methyltransferase (COG0500), conserved archaeal protein (COG3373)
PAB1817-PAB2392	12	6						(Lhr-like helicase (COG1201), cell division GTPase COG0206), uncharacterized conserved protein COG5306), uncharacterized membrane protein (COG4505)
<b><i>Pyrococcus furiosus</i> DSM 3638</b>								
PF0340-PF0329	12	2	✓	✓	✓			uncharacterized archaeal protein (COG3373), SAM dependent methyltransferase (COG0500)
PF0472-PF0469	4	1					✓	uncharacterized conserved proteins (COG4043/COG2411)
PF1307-PF1303	5	2						
PF1504-PF1511	8	2						Lhr-like helicase (COG1201), cell division GTPase (COG0206)
<b><i>Pyrococcus horikoshii</i> OT3</b>								
PH0544-PH0560	14	5	✓	✓	✓			uncharacterized archaeal protein (COG3373), SAM dependent methyltransferase (COG0500)
PH1329-PH1344	12	5						Lhr-like helicase (COG1201), cell division GTPase (COG0206), uncharacterized protein (COG5306), uncharacterized membrane protein (COG4505)
<b><i>Thermococcus kodakaraensis</i> KOD1</b>								
TK0036-TK0050	13	5	✓	✓	✓			
TK1670-TK1661	10	5						Lhr-like helicase (COG1201), cell division GTPase (COG0206)
TK1707-TK1695	11	2						
<b><i>Thermoplasma acidophilum</i> DSM 1728</b>								
Ta0553-Ta0556	4	1	✓					
<b><i>Thermoplasma volcanium</i> GSS1</b>								
TVN0607-TVN0614	8	1	✓	✓	✓			

<b><i>Nanoarchaeum equitans</i> Kin4-M</b>								
NEQ137-NEQ132	5	2						

\*flagellar accessory genes

†substrate(s) in these operons were included after reannotation: VNG0961G, VNG0962G and VNG1009G (missing leader), MJ1467 (long leader), MMP1666 (missing leader), MTH422 (long leader), PF0338 (missing leader), Saci\_1496.1 (not annotated as gene)

**Table S3. Plasmids used in this study**

Plasmid	Description	Source
pBAD/Myc-His A	<i>E. coli</i> expression vector, arabinose inducible promoter	Invitrogen
pZA7	T7 expression vector, C-terminal HA tag	Ref x
pSA4	T7 expression vector, C-terminal 6xHIS tag	Ref x
pZA10	pZA7 with <i>epdA</i>	This study
pZA11	pZA7 with <i>epdB</i>	This study
pZA12	pZA7 with <i>flaB2</i>	This study
pZA13	pSA4 with <i>eppA</i>	This study
pZA14	pSA4 with <i>flaK</i>	This study
pZA10- <i>eppA</i>	<i>epdA/eppA</i> co-expression	This study
pZA10- <i>flaK</i>	<i>epdA/flaK</i> co-expression	This study
pZA10- <i>pibD</i>	<i>epdA/pibD</i> co-expression	This study
pZA11- <i>eppA</i>	<i>epdB/eppA</i> co-expression	This study
pZA11- <i>flaK</i>	<i>epdB/flaK</i> co-expression	This study
pZA11- <i>pibD</i>	<i>epdB/pibD</i> co-expression	This study
pZA12- <i>eppA</i>	<i>flaB2/eppA</i> co-expression	This study
pZA12- <i>flaK</i>	<i>flaB2/flaK</i> co-expression	This study
pZA12- <i>pibD</i>	<i>flaB2/pibD</i> co-expression	This study
pZA13	pZA7- <i>epdA</i> (RGQI → KGAS)	This study
pZA14	pZA7- <i>epdB</i> (KGQV → KGAS)	This study
pZA15	pZA7- <i>flaB2</i> (KGAS → RGQI)	This study
pZA13- <i>eppA</i>	<i>epdA<sup>KGAS</sup>/eppA</i> co-expression	This study
pZA13- <i>flaK</i>	<i>epdA<sup>KGAS</sup>/flaK</i> co-expression	This study
pZA14- <i>eppA</i>	<i>epdB<sup>KGAS</sup>/eppA</i> co-expression	This study
pZA14- <i>flaK</i>	<i>epdB<sup>KGAS</sup>/flaK</i> co-expression	This study
pZA15- <i>eppA</i>	<i>flaB<sup>RGQI</sup>/eppA</i> co-expression	This study
pZA15- <i>flaK</i>	<i>flaB<sup>RGQI</sup>/flaK</i> co-expression	This study
pZA16	pZA7- <i>edpA</i> -6xHIS	This study
pZA17	pZA7- <i>edpB</i> -6xHIS	This study
pZA18	pZA7- <i>flaB2</i> -6xHIS	This study
pZA16- <i>eppA</i>	<i>edpA</i> -6xHIS/ <i>eppA</i> co-expression	This study
pZA17- <i>eppA</i>	<i>edpB</i> -6xHIS/ <i>eppA</i> co-expression	This study
pZA18- <i>flaK</i>	<i>flaB2</i> -6xHIS/ <i>flaK</i> co-expression	This study

